Magnetically Aligned Bicelles To Study the Orientation of Lipophilic Ligands in Membrane Bilayers

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Magnetically aligned bicelles were used as a model membrane to study the orientation and dynamic properties of two cannabinoids (Δ^8-THC and Me-Δ^8-THC) using ^{31}P and ^{2}H NMR. The uniform alignment of the bicelles allowed us to obtain well resolved deuterium spectra from a solution NMR spectrometer. The preferred orientations of Δ^8-THC and Me-Δ^8-THC were calculated on the basis of the measurements of individual quadrupolar splittings. Our results agree with previous experiments using multilamellar membranes as well as with molecular dynamics simulation data described here. In conjunction with our earlier report using small and fast tumbling bicelles, the present work of well aligned bicelles shows that bicelle preparations can provide either pseudoisotropic or anisotropic NMR spectra to study the conformation, orientation, and dynamic properties of ligands in membrane bilayers. Such data are of critical value for understanding the interactions of lipophilic drug molecules with membrane proteins.

Introduction

The phospholipid bilayer membrane represents many of the basic structural and functional features of cell membranes. Multilamellar vesicles (MLV) have been used extensively as model membrane preparations for solid-state NMR experiments.[1−3] In such preparations in which ^{13}C, ^{2}H, or ^{31}P labels are used, the molecules are randomly dispersed with respect to the direction of the magnetic field and produce broad "powder pattern" spectra. Improvement in spectral resolution can be obtained using multilamellar vesicles that are sandwiched between glass plates, thus producing uniformly aligned membrane systems.[4,5] More recently, phospholipid bicelles have been increasingly used to obtain conformational and dynamic information on molecules that associate with membrane surfaces[6−9] as well as peptides and proteins that span the bilayer.[10−14] While the above studies involved flexible molecules, the focus of this work is on rigid drug analogues for which axial symmetry cannot be assumed a priori.

Under proper conditions, a bicelle system can provide either pseudoisotropic or anisotropic NMR spectra, a unique feature that is not available in other model membrane systems. Earlier, we have reported how a pseudoisotropic disk-shaped bicelle preparation can be used to study the conformations of two lipophilic cannabinoids using high-resolution NMR through the measurements of intramolecular NOE values.[15,16] In this communication, we exploit the uniform alignment of bicelles for studying the orientation and dynamic properties of lipophilic drug molecules in an anisotropic membrane system. To validate our system, we have determined the preferred orientations of two earlier studied cannabinoids with respect to the bilayer normal based on measurements of residual quadrupolar splittings from their ^{2}H spectra. Our new data are congruent with earlier results[17] using stationary solid state ^{2}H NMR with a multilamellar membrane preparation.

Bicelles are extremely versatile because their overall morphology and dynamic properties can be appropriately adjusted by varying the values of lipid-to-detergent ratio (q), total lipid concentration, buffer pH, ionic strength, and temperature.[6] A typical bicelle preparation is composed of carefully selected ratios of a long acyl chain lipid such as 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC[a]) in combination with a detergent such as 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) or a bile-salt derivative 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propane sulfonate (CHAPSO). When in an aqueous suspension, this lipid combination spontaneously assembles into disk-shaped bilayer membranes. The DMPC-rich domains of the bicelles exhibit phase transitions between the liquid crystalline (L_α) and the gel (L_β) phases, as in a multilamellar vesicle (MLV) bilayer. A series of titration studies demonstrated[6] that the liquid crystalline L_α DMPC/DHPC bicelles with q ranging from 2.0 to 3.5 are aligned with their bilayer normal perpendicular to the external magnetic field. Furthermore, the addition of KCl can drastically improve the degree of alignment.[18] The formation of magnetically aligned phospholipid bicelles under various conditions and their corresponding properties have been extensively reviewed.[14,19,20]

We have now incorporated (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC) or *O*-methyl-(−)-Δ^8-tetrahydrocannabinol (Me-Δ^8-THC) into a bicelle system with a lipid concentration of 25% (w/v) and a DMPC/DHPC ratio of $q = 2.7$. The restricted magnetic alignment of the bicelles allowed us to record well-resolved ^{31}P and ^{2}H NMR spectra. ^{31}P NMR was used to monitor any morphological changes of the bicelle disk after incorporation of the cannabinoid ligands. ^{2}H NMR quadrupolar splittings from strategically ^{2}H-labeled Δ^8-THC and Me-Δ^8-THC were used to determine the preferred ligand orientation in a bilayer environment. Our results are supported by molecular dynamics simulations of these two ligands within a DMPC lipid bilayer model. The congruence between findings of this study and those from previous work using multilamellar bilayer demonstrates that the bicelle system is a suitable membrane model and can be used to obtain

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[a] Abbreviations: Δ^8-THC, (−)-Δ^8-tetrahydrocannabinol; Me-Δ^8-THC, *O*-methyl-(−)-Δ^8-tetrahydrocannabinol; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

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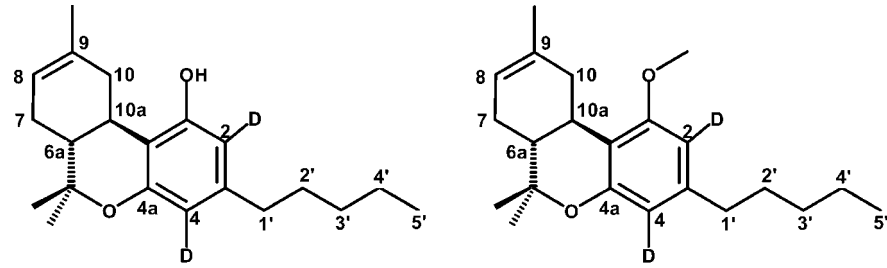

Figure 1. Structures of (−)-Δ^8-tetrahydrocannabinol (Δ^8-THC) and *O*-methyl-(−)-Δ^8-tetrahydrocannabinol (Me- Δ^8-THC) showing the positions of deuterium labels.

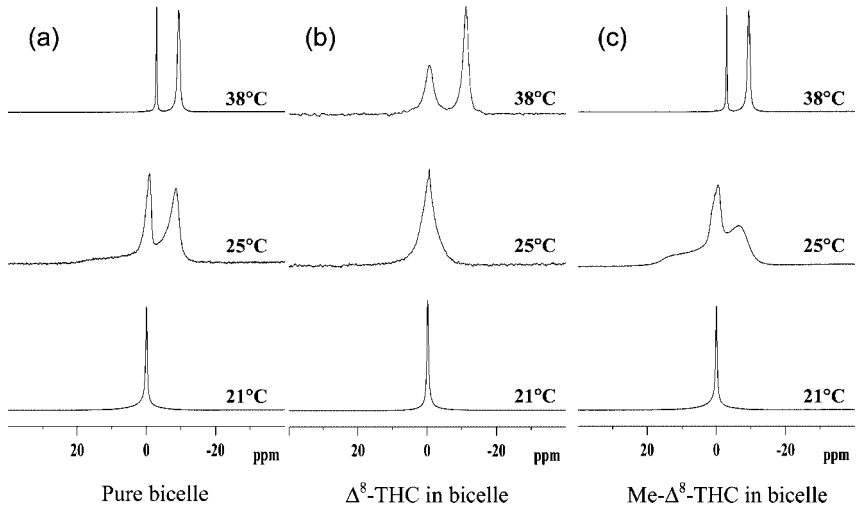

Figure 2. ^{31}P NMR spectra of (a) DMPC/DHPC (2.7:1) bicelle solution at a total lipid concentration of 25% (w/v) and with the incorporation of (b) Δ^8-THC or (c) Me-Δ^8-THC. At 38 °C, the two sharp peaks have an integration ratio of 2.7:1, clearly indicating that the bicelles are intact and well aligned with its bilayer normal perpendicular to the magnetic field.

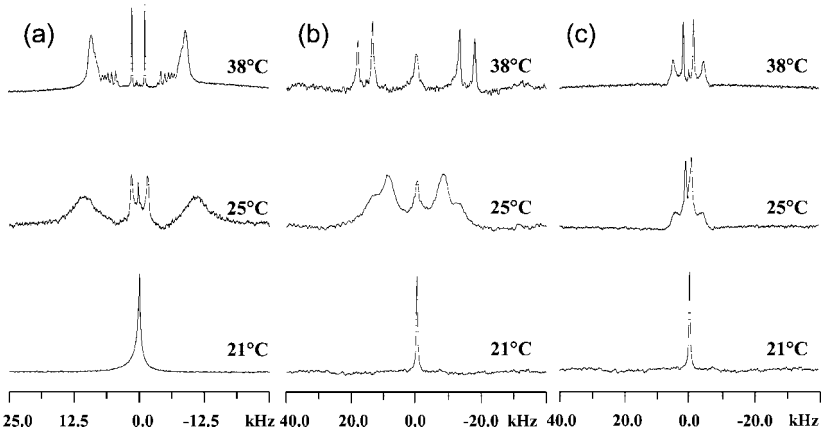

Figure 3. Comparison of ^2H NMR spectra of a 25% (w/v) bicelle preparation containing (a) 10% acyl chain perdeuterated DMPC, (b) [2,4-$^2H_2$]Δ^8-THC, and (c) [2,4-$^2H_2$]Me-Δ^8-THC.

either isotropic or anisotropic NMR spectra for studying interactions between ligands and the membrane bilayer.

Materials and Methods

Materials. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) were purchased from Avanti Polar Lipids (Alabaster, AL). [2,4-$^2H_2$]Δ^8-THC and [2,4-$^2H_2$]Me-Δ^8-THC (Figure 1) were synthesized in our laboratory.[21] All ^{13}P and ^2H NMR samples were prepared by the same procedure as described briefly below. [2,4-$^2H_2$]Δ^8-THC or [2,4-$^2H_2$]Me-Δ^8-THC, DMPC, and DHPC were mixed in chloroform (99% pure, Aldrich, Milwaukee, WI), which was then evaporated using an N_2 stream. The sample was dried under vacuum overnight. Bicelles were prepared by transferring the powder mixture directly into a 5 mm NMR tube and adding an appropriate amount of 0.1 M KCl solution in deuterium-depleted water (deuterium 2−3 ppm, Cambridge Isotope Laboratory, Andover, MA). The preparation then underwent a combination of mechanical blending, heating, and cooling until a clear and homogeneous system was obtained. All bicelle samples contain DMPC and DHPC with a molar ratio of $q = 2.7$, a total lipid concentration of 25% (w/v), and a molar concentration of 10% Δ^8-THC or Me-Δ^8-THC relative to DMPC.

NMR Experiments. All ^{31}P and ^2H NMR experiments were carried out on a Bruker DRX-500 solution NMR spectrometer using

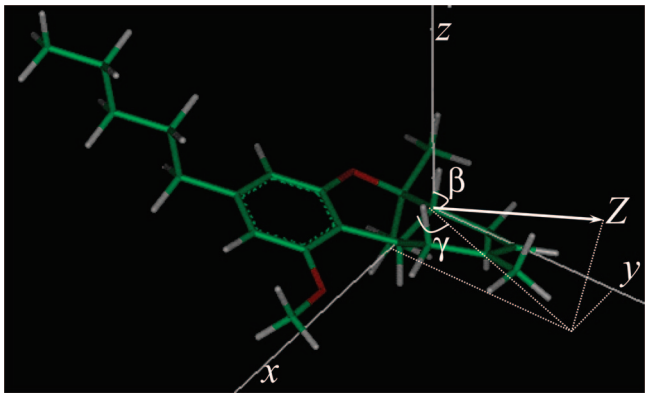

Figure 4. Molecular fixed coordinate system used for calculating the preferred drug orientation in membrane bilayer where the *Z*-axis represents the bilayer normal direction. The origin is at atom C(6a), the *x*-axis is along the C(6a)−C(10a) bond, and the *z*-axis is in the plane of atoms C(6a), C(10a), and H(6a).

Table 1. Direction Cosines and Quadrupolar Splittings of C−^{2}H Bonds in [2,4-^{2}H$_2$]Δ^8-THC and [2,4-^{2}H$_2$]Me-Δ^8-THC

				observed $\Delta\nu_Q$ (kHz)			
				Δ^8-THC		Me-Δ^8-THC	
deuteron position	cos α_x	cos α_y	cos α_z	in bicelle	in MLV	in bicelle	in MLV
2	0.957	−0.075	0.281	21.0	23.0	4.2	4.9
4	−0.570	−0.821	−0.001	31.4	34.6	14.1	22.8

a 5 mm broadband direct-observe probe. Proton decoupled ^{31}P spectra were recorded in order to monitor any morphological changes of the bicelle disk in bicelle preparations with and without the strategically deuterated ligands [2,4-^{2}H$_2$]Δ^8-THC or [2,4-^{2}H$_2$]Me-Δ^8-THC. The ^{2}H NMR experiments were conducted on the broadband channel tuned at 76.753 MHz while blanking the lock channel. A quadrupolar echo pulse sequence was used to obtain the free induction decay (FID) with a 45 μs duration between two 90° pulses of 8.9 μs. Orientations of the Δ^8-THC and Me-Δ^8-THC in the bicelle system were determined on the basis of the quadrupolar splitting values using a complete order parameter tensor approach developed earlier in our laboratory.[17,22] By examination of the root-mean-square deviation (rmsd) between the experimentally observed quadrupolar splittings and the calculated splitting values, the resulting Euler angles β and γ with the minimum rmsd represent the direction of the bilayer normal in the molecular-fixed coordinate system, thus allowing us to determine the preferred orientations of these cannabinoids in the membrane bilayer.

Molecular Dynamics Simulations. Molecular dynamics (MD) simulations were performed with the GROMACS 3.2.1 software package installed on a Dell PowerEdge 6600 parallel computing server running Red Hat Enterprise Linux Advanced Server, version 2.3 (Dell, Inc., Round Rock, TX). MD simulation parameters were based upon studies by Falck et al.[23] and Patra et al.[24] A DMPC bilayer model containing a total of 128 phospholipid molecules and 3655 water molecules (dmpc_npat.pdb) and the corresponding topology (dmpc.itp) file were obtained from the University of Calgary's biocomputing Web server.[25] The DMPC bilayer model, complete with solvent box coordinates and water molecules, was first modified by replacing one DMPC molecule with either Δ^8-THC or Me-Δ^8-THC. The system was minimized with the steepest descent algorithm until the maximum derivative was less than 100 kcal/(mol·Å). Molecular dynamics simulations were then carried out for 2000 ps with a time step of 2 fs, and conformations were recorded every 2000 steps for a total of 500 trajectories. Periodic boundary conditions were applied during the entire dynamics simulations. The particle-mesh Ewald (PME) method[26] was used to calculate the long-range electrostatic interactions, where interactions within 1.0 nm were calculated at each time step and those beyond this range were determined every 10 steps. Semi-isotropic pressure coupling was achieved using the Berendsen algorithm[27] with a time constant of 1 ps for both the *xy*-plane and the *z*-axis. During the dynamics calculations, all components of the system were separately coupled to a heat bath at 38 °C using the Berendsen algorithm with a time constant of 0.1 ps.

Results and Discussion

Bicelle Morphology Studies by ^{31}P NMR. Figure 2 shows the ^{1}H-decoupled ^{31}P NMR spectra from bicelle preparations without and with Δ^8-THC or Me-Δ^8-THC at increasing temperatures. At 21 °C, a single sharp peak is observed for all preparations. As the temperature is increased to 25 °C, the bicelle samples start to undergo a phase transition that alters the DMPC dynamics and/or the bicelle disk alignment. This phase transition is reflected in the ^{31}P spectra as follows: (a) two distinct broadened peaks for pure bicelle; (b) a broadened resonance due to overlapping signals from DMPC and DHPC for bicelles with Δ^8-THC; and (c) transformation of the DMPC peak into a typical chemical shift anisotropy line shape for bicelles with Me-Δ^8-THC.

However, at 38 °C all these preparations exhibit similar general spectral features characteristic of intact bicelle preparations. The pure bicelles exist in the liquid crystalline phase and become magnetically aligned, as reflected by the two distinct sharp peaks in the ^{31}P spectrum (Figure 2a). The peak at −3.0 ppm is due to the short acyl chain DHPC, while the peak at −9.1 ppm corresponds to the long acyl chain DMPC. These values are consistent with results from a detailed bicelle alignment study,[28] which confirms that the bicelle disk is aligned with its bilayer plane parallel to the external magnetic field. Integration of these two peaks shows that they have an expected intensity ratio of 2.7:1, precisely equal to the ratio (q) of DMPC to DHPC in the preparation. The bicelle preparations into which the two cannabinoid ligands were incorporated at 38 °C also exhibit two peaks in their ^{31}P NMR spectra (Figure 2b,c) with the same ratio of 2.7:1, suggesting that the presence of Δ^8-THC or Me-Δ^8-THC does not disrupt the morphology and alignment of the bicelles at this temperature. Upon closer examination, we observe distinct differences in the spectra from the two cannabinoid preparations. In the Δ^8-THC spectrum, both peaks are broadened and the DMPC resonance is shifted upfield. Conversely, Me-Δ^8-THC produces no discernible changes in the bicelle spectra. These observations are congruent with the results of earlier ^{2}H NMR[17] and ^{13}C NMR studies.[29]

The ^{31}P spectra suggest that Δ^8-THC and Me-Δ^8-THC interact differently with the bicelles, but neither molecule disrupts the integrity or alignment of the bicelles within the magnetic field. The data are consistent with previous DSC and solid-state NMR studies of these two ligands interacting with DPPC model membrane bilayers.[17]

Stationary ^{2}H NMR. The orientation of lipophilic ligands in a membrane system can be studied from their solid-state ^{2}H NMR spectra in multilamellar membrane preparations. Such spectra are obtained using specifically designed wideline probes coupled with high power amplifiers. Conversely, in our membrane preparation, as the bicelle disks are very well aligned within the magnetic field, we expect to observe sharp deuterium resonances rather than a powder spectrum. Furthermore, the residual quadrupolar splittings from the more mobile bicelle samples are generally smaller than those in multilamellar vesicles. These sharp deuterium resonances can therefore be observed on a standard solution NMR instrument equipped with a relatively low-power amplifier. Indeed, all our deuterium experiments on magnetically aligned bicelle samples were performed on a Bruker DRX500 solution NMR spectrometer.

Figure 3 shows ^{2}H spectra of a bicelle preparation containing (a) 10% of DMPC with perdeuterated acyl chains, (b) [2,4-$^2H_2$]Δ^8-THC, and (c) [2,4-$^2H_2$]Me-Δ^8-THC. As the temperature is reduced from 38 to 21 °C, the spectrum of pure bicelles (sample a) undergoes typical changes reflecting the bilayer phase transitions. Parallel experiments with nondeuterated bicelles containing each of the two cannabinoids deuterated in the 2 and 4 positions (samples b and c) exhibit analogous temperature related changes, suggesting that the two lipophilic ligands are fully incorporated into the membrane bilayer system while in the liquid crystalline phase. This confirms our observation with the ^{31}P spectra in which neither Δ^8-THC nor Me-Δ^8-THC disrupts the bilayer structure within the bicelle. At 38 °C, the ^{2}H spectrum due to [2,4-$^2H_2$]Δ^8-THC bicelle preparation exhibits two sharp doublets with a quadrupolar splitting of $\Delta\nu_Q = 21.0$ and 31.4 kHz. These were assigned to 2 and 4 positions, respectively, by analogy to ^{2}H spectra from multilamellar preparations.[17] At the same temperature, the spectra due to [2,4-$^2H_2$]Me-Δ^8-THC in bicelles also exhibit two sharp doublets, however, with smaller quadrupolar splitting values of $\Delta\nu_Q = 4.2$ and 14.1 kHz for the 2 and 4 positions, respectively. We recognize that our ligands may reside in both the bilayer and the curved regions of the bicelle. The former would be expected to exhibit a spectrum with sharp doublets. Conversely, spectra from the curved regions, because of lack of alignment, would be broad Pake patterns. The spectra we obtained in our experiments clearly argue that they are due to alignment of the deuterated ligands within the bilayer region, and the sharp doublets are not shifted by any presence of broad Pake patterns.

In an earlier work,[17] we have observed analogous differences in ^{2}H quadrupolar splittings in which multilamellar vesicles were utilized. This striking difference in the spectral properties of Δ^8-THC and Me-Δ^8-THC when studied in similar membrane preparations was attributed to differences in the manner in which each of the lipophilic ligands orients within the anisotropic membrane system. We used the values of these ^{2}H quadrupolar splittings to determine the orientation of a drug molecule in the membrane.

Calculation of the Preferred Ligand Orientation in Bicelles. The quadrupolar splitting from an ^{2}H label within a ligand depends on the angle between the particular C$-^2$H bond and the director axis of membrane bilayer. In its liquid crystalline phase, the bilayer allows the drug molecule to undergo anisotropic motions, which is described by an order parameter tensor **S** with a principal *Z*-axis along the director axis (perpendicular to the bilayer plane and essentially parallel to the lipid acyl chains). NMR quadrupolar interaction theory gives the following relationship between the observed ^{2}H quadrupolar splitting $\Delta\nu_Q$ and the order parameter tensor **S**:[30]

$$\Delta\nu_Q = \frac{3}{4}A_Q \sum S_{ij}\cos\alpha_i\cos\alpha_j \quad (1)$$

where A_Q is the quadrupolar coupling constant (180 kHz for aromatic deuterons[31]), i and j represent the x, y, or z axes that are fixed on the ligand molecule, and cos α_x, cos α_y, and cos α_z are the direction cosines of the C$-^2$H bond in this ligand-fixed coordinate system. In the principal axis system (or bilayer-fixed coordinate system) where the order parameter tensor only contains its principal elements (S_{XX}, S_{YY}, and S_{ZZ}), this relationship can be simplified to

$$\Delta\nu_Q = \frac{3}{4}A_Q(S_{XX}\cos^2\alpha_X + S_{YY}\cos^2\alpha_Y + S_{ZZ}\cos^2\alpha_Z) \quad (2)$$

where cos α_X, cos α_Y and cos α_Z are the direction cosines of the C$-^2$H bond with respect to the principal *X*, *Y*, and *Z* axes. When several $\Delta\nu_Q$ values are available, we can determine the order parameter tensor **S** and deduce how the ligand orients with respect to the principal *Z*-axis of the bilayer (in terms of two Euler angles β and γ). β is the angle between the bilayer's *Z*-axis and the ligand's *z*-axis, and γ is the angle between the *xy*-projection of bilayer's *Z*-axis and the ligand's *x*-axis.

We used the same choices of ligand-fixed coordinate system as in our earlier calculations:[17] the origin at atom C(6a), the *x*-axis along the C(6a)−C(10a) bond, and the *z*-axis in the plane of atoms C(6a), C(10a), and H(6a) (Figure 4). The molecular structures were derived from X-ray crystallographic data of (−)-Δ^9-tetrahydrocannabinolic acid[32] and are consistent with the tricyclic ring conformation of (−)-Δ^9-THC as determined by high-resolution NMR.[33] Table 1 lists the direction cosines (cos α_x, cos α_y, and cos α_z) of the C$-^2$H bond vectors (2- and 4-positions) in the ligand-fixed coordinate system and the observed quadrupolar splitting values for both Δ^8-THC and Me-Δ^8-THC in bicelle and multilamellar membrane bilayers, respectively. Inspection of the ^{2}H spectra from the bicelle preparations of Δ^8-THC and Me-Δ^8-THC in Figure 3 reveals certain similarities to their MLV counterparts except that the quadrupolar splittings in bicelle preparations are consistently narrower, reflecting the higher overall mobility of the individual ligands and/or bicellar membranes. This may be attributed, at least in part, to wobbling motions as previously observed for bicelles at low q values ($q < 3.0$).[14] In our experiments we have strategically introduced all ^{2}H labels within the same rigid tricyclic component of the tetrahydrocannabinol structure. We thus expect that additional motions described above would affect the dynamics of all C$-^2$H bonds equally. For this reason, the observed differences in ^{2}H splittings can be attributed to the orientation of individual C$-^2$H bonds with respect to the direction of the long lipid acyl chain.

Our calculation takes into consideration all the motional effects to which the ligands are subjected by introducing an overall correction factor into the new order parameter tensor ($\mathbf{S}_{\mathbf{bicelle}}$). This correction factor was obtained by scaling down the principal components of the order-parameter tensor ($\mathbf{S}_{\mathbf{MLV}}$) for Δ^8-THC or Me-Δ^8-THC that we have previously obtained from DPPC MLV preparations.[17] For Δ^8-THC, a scaling factor of 0.90 was obtained from the average ratios of the corresponding quadrupolar splitting values, and the new order parameter tensor ($\mathbf{S}_{\mathbf{bicelle}}$) for Δ^8-THC in the principal axis system became

$$\mathbf{S}_{\mathbf{bicelle}} = 0.90 \times \begin{pmatrix} -0.48 & 0 & 0 \\ 0 & -0.12 & 0 \\ 0 & 0 & 0.60 \end{pmatrix}$$

$$= \begin{pmatrix} -0.43 & 0 & 0 \\ 0 & -0.11 & 0 \\ 0 & 0 & 0.53 \end{pmatrix} \quad (3)$$

Similarly, for Me-Δ^8-THC the scaling factor was determined to be 0.74, which gave the new order parameter tensor ($\mathbf{S}_{\mathbf{bicelle}}$) for Me-$\Delta^8$-THC in the principal axis system as

$$\mathbf{S}_{\mathbf{bicelle}} = 0.74 \times \begin{pmatrix} -0.38 & 0 & 0 \\ 0 & -0.03 & 0 \\ 0 & 0 & 0.41 \end{pmatrix}$$

$$= \begin{pmatrix} -0.27 & 0 & 0 \\ 0 & -0.02 & 0 \\ 0 & 0 & 0.29 \end{pmatrix} \quad (4)$$

For each possible combination of β and γ angles, we calculated the direction cosines (cos α_X, cos α_Y, and cos α_Z) of the C$-^2$H bonds in the principal axis system, and we used eq 2 to obtain theoretical quadrupolar splittings ($\Delta\nu_Q$). These $\Delta\nu_Q$ values were then compared with the experimental results, and the overall

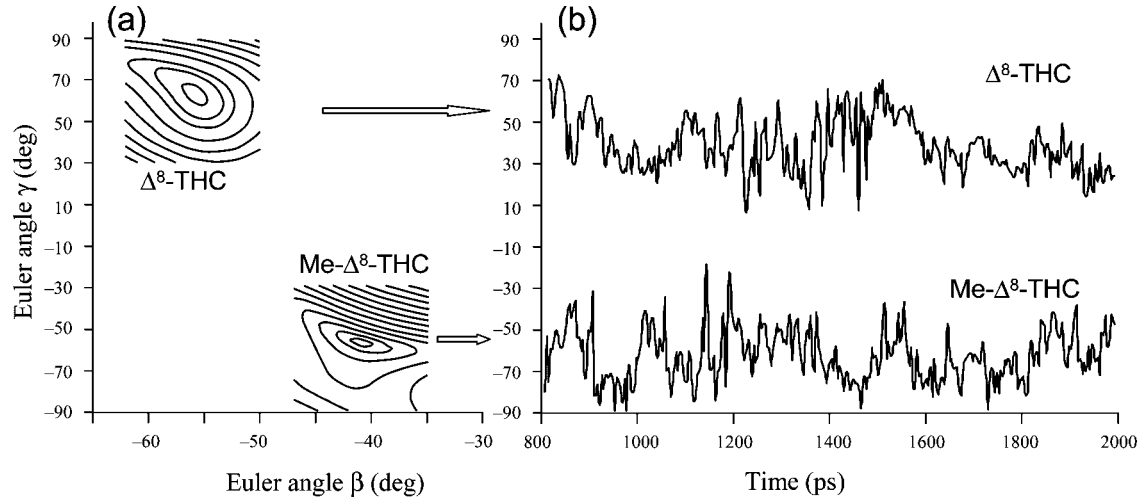

Figure 5. (a) Contour plots of root-mean-square deviation (rmsd) between the calculated and observed quadrupolar splittings from [2,4-$^2H_2$]Δ^8-THC and [2,4-$^2H_2$]Me-Δ^8-THC in bicelles, graphed against various combinations of Euler angles β and γ. For Δ^8-THC, the lowest rmsd is 0.04 kHz and the contour level values are 0.05, 0.10, 0.15,..., 0.45 kHz (from the innermost to the outermost). For Me-Δ^8-THC, the lowest rmsd is 0.01 kHz and the contour level values are 0.10, 0.20, 0.30,..., 1.30 kHz (from the innermost to the outermost). (b) Euler angle γ from molecular dynamics simulations of Δ^8-THC and Me-Δ^8-THC in DMPC bilayers. A comparison of (a) and (b) shows good agreement between the γ values from ^{2}H NMR experiments and those from molecular dynamics simulations.

Table 2. Orientations of Δ^8-THC and Me-Δ^8-THC in DMPC/DHPC Bicelle and DPPC MLV Bilayer, As Represented by the Euler Angles β and γ

	orientation (deg)			
	in DMPC/DHPC bicelle		in DPPC MLV bilayer	
cannabinoid	β	γ	β	γ
Δ^8-THC	−56	+64	−57	+50
Me-Δ^8-THC	−41	−58	−37	−66

difference was indicated by the root-mean-square deviation (rmsd). Contour graphs of rmsd are presented in Figure 5a, where the rmsd minimum represents the best match in each case. For Δ^8-THC the rmsd minimum occurs at $\beta = -56°$ and $\gamma = 64°$, whereas for Me-Δ^8-THC it occurs at very different values of the Euler angles ($\beta = -41°$ and $\gamma = -58°$). As we can see in Table 2, for each ligand where these values are compared with their corresponding Euler angles in DPPC MLV bilayers, the β-values are almost identical (discrepancy of $\leq 4°$) and the γ-values are also similar (discrepancy of $\leq 14°$). Comparing the Euler angles between the two ligands, we found a major difference of 122° in their γ-values. It is mainly the γ angle that distinguishes their orientations: Δ^8-THC orients in the membrane with the long axis of its tricyclic ring system almost perpendicular to the lipid acyl chains, whereas Me-Δ^8-THC has its long axis nearly parallel to the lipid acyl chain direction.

As described in our MLV work,[17] the "awkward" orientation of Δ^8-THC places its phenolic hydroxyl group at the bilayer polar interface and extends its hydrophobic moiety into the lipid acyl chain region. The phenolic −OH group serves as an anchoring point, and this may explain the stronger perturbation effects of Δ^8-THC observed in the ^{31}P NMR spectra. Our calculations show that Me-Δ^8-THC assumes an orientation parallel to the bilayer acyl chains and only perturbs the bilayer minimally. Although Me-Δ^8-THC differs structurally from Δ^8-THC only by a methyl ether group replacing the phenolic hydroxyl group, the drastic difference in their orientations in the membrane indicates that they have very different interactions with membrane bilayers.

Molecular Dynamics Simulations. To further confirm our experimental findings, we used molecular dynamics simulations to incorporate each of the two cannabinoids into a membrane bilayer model of 128 DMPC molecules and 3655 water molecules.[25] The following partial charges were placed after converting the coordinates to a topology format:[34,35] for Δ^8-THC, C1 = 0.14, C1 oxygen = −0.54, C1-OH hydrogen = 0.40, C4a = 0.18, C4a-pyran oxygen = −0.36, and C5 = 0.18; for Me-Δ^8-THC, C1 = 0.16, C1-methoxy oxygen = −0.36, C1-methoxy carbon = 0.20, C4a = 0.18, C4a-pyran oxygen = −0.36, and C5 = 0.18. All other atoms were electrically neutral. Irrespective of the anticipated differences in their orientations, we initially placed Δ^8-THC and Me-Δ^8-THC in an identical orientation and position in the bilayer before the start of dynamics simulation. Both ligands were placed at the bilayer−water interface, with their long axis parallel to the bilayer normal. Additionally, all angular and distance restraints were completely relaxed during the dynamics simulation, in order to fully sample all possible orientations of the two ligands. A total of 500 conformations for each molecule were recorded during a total of 2000 ps of simulation time and led to information on their orientations and locations within the bilayer.

At the beginning of the dynamics simulation, the center of mass for both ligands was placed at 8.5 Å from the bilayer center (corresponding to the bilayer−water interface). Figure 6a demonstrates how the location of ligand's center of mass migrates within the DMPC bilayer membrane during the 2000 ps of simulated time for Δ^8-THC and Me-Δ^8-THC. After about $t = 800$ ps, Me-Δ^8-THC starts to sink into the center of the DMPC bilayer and resides at the bilayer hydrocarbon core with its center of mass around 4.0 ± 2.5 Å from the bilayer center. On the other hand, Δ^8-THC remains at the bilayer−water interface during the entire time of our dynamics simulation as shown in Figure 6b, a dynamic behavior that can be attributed to intermolecular H-bondings between the phenolic hydroxyl group on Δ^8-THC and the phosphate of DMPC. However, substitution of the phenolic hydroxyl with a methoxy group precludes the interaction of the −OH hydrogen with the heteroatom of the phospholipid headgroup. As a result, Me-Δ^8-THC prefers to reside deeper within the hydrophobic region of the bilayer.

After the initial 800 ps equilibration, Δ^8-THC and Me-Δ^8-THC begin to assume their distinct orientations that they maintain during the remaining period of the dynamics simulations. Figure 5b shows the Euler angle γ of the 300 conformations of each molecule for simulation time from $t = 800$ ps to $t = 2000$ ps. The two curves

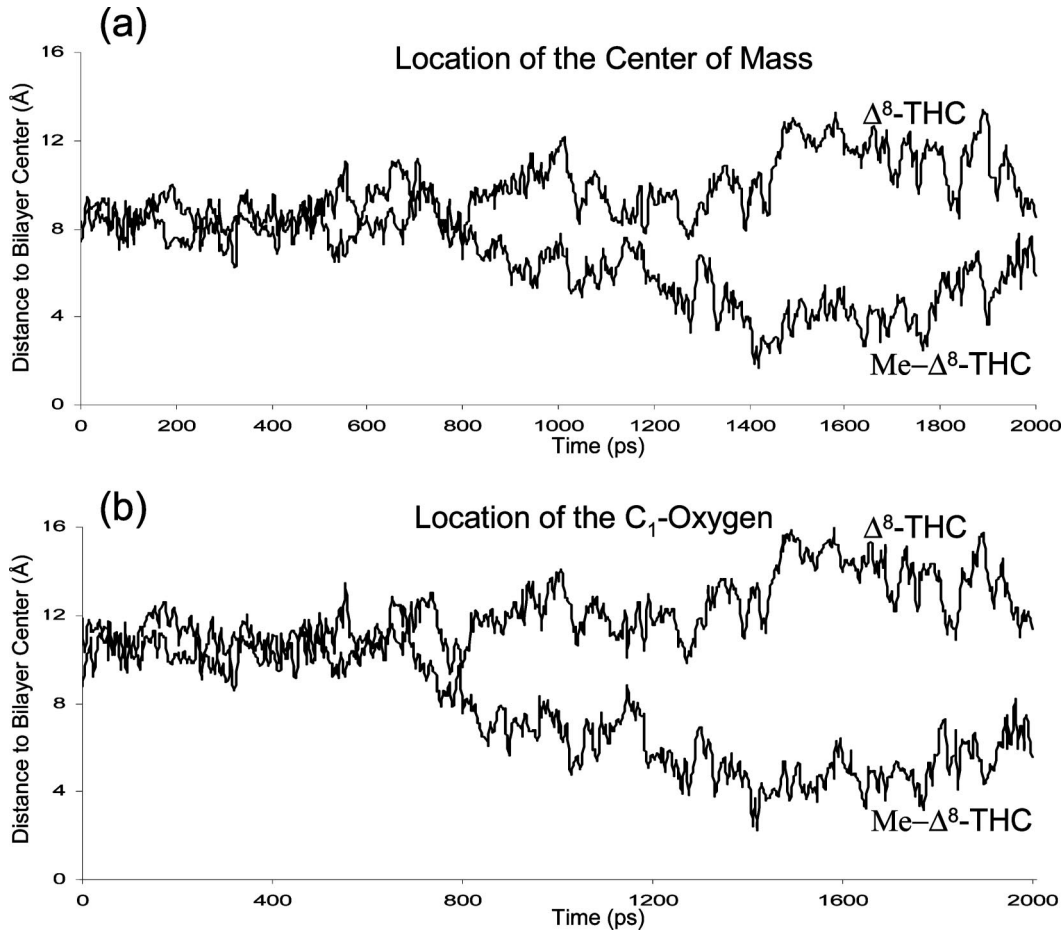

Figure 6. (a) Location of the center of mass of Δ^8-THC and Me-Δ^8-THC in DMPC membrane bilayer during the 2000 ps of simulated time. (b) Location of the phenolic oxygen of Δ^8-THC and Me-Δ^8-THC in DMPC membrane bilayer.

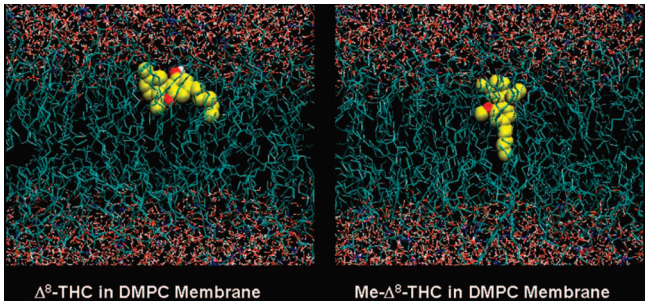

Figure 7. Two representative orientations of Δ^8-THC and Me-Δ^8-THC in DMPC bilayer derived from molecular dynamics simulations.

are clearly separated, each curve showing reasonable fluctuations and deviations from its statistical average. Comparing the respective contour graph for each molecule in Figure 5a to the left, we can see a good agreement between the simulated result and its experimentally determined orientation. As discussed above, the distinction between the orientations of Δ^8-THC and Me-Δ^8-THC is most aptly represented by the γ value, the angle between the projection of the bilayer normal in the *xy*-plane and the *x*-axis along the C(6a)−C(10a) bond. Among these 300 snapshots, the average γ is $42 \pm 20°$ for Δ^8-THC and $-62 \pm 20°$ for Me-Δ^8-THC, where we can see a large difference of 104° in their γ values. Two representative orientations (one for Δ^8-THC and one for Me-Δ^8-THC, in simulated DMPC bilayers) are depicted in Figure 7.

Conclusions

We have previously proposed that the proper conformation, orientation, and location of a drug molecule in the membrane are critical for it to reach its site of action and interact productively with that site.[15,36−38] In this work, two lipophilic cannabinoids, Δ^8-THC and Me-Δ^8-THC, were successfully incorporated into a magnetically aligned phospholipid bicelle membrane system and ^{31}P NMR spectra have demonstrated that neither the bilayer structure nor the bicelle alignment is disrupted. ^{2}H NMR experiments confirmed that both cannabinoid ligands reside within the bicelle bilayer and that their dynamic properties are congruent with those of the DMPC acyl chains. We have accurately measured the deuterium quadrupolar splittings from strategically ^{2}H-labeled cannabinoids and used them to calculate the preferred orientations of these ligands within the bicelles. We found that Δ^8-THC orients with the major axis of its tricyclic ring system perpendicular to the bilayer normal, while Me-Δ^8-THC orients parallel with the phospholipid acyl chains.

We conclude that magnetically aligned bicelles provide the following unique experimental advantages for investigating the preferred ligand orientation in a lipid bilayer environment. Because of the uniform alignment of bicelles, we were able to obtain well-resolved ^{2}H NMR spectra using a solution NMR spectrometer, whereas membrane bilayer preparations usually require the use of solid-state NMR spectrometers equipped with wideline probes and high power amplifiers. Further, these experiments can be conducted with a relatively small amount of sample and yield sharper spectra that can provide more accurate measurements of quadrupolar splittings. It is noted that we were able to obtain our orientational

information by using only two deuterium labels because we had adequate knowledge of the properties of these two molecules in a membrane environment from previous work.[17] With ligands for which such information is not available, this matrix/tensor approach would require six deuterium labels on the rigid part of the molecule.

In conjunction with our earlier report in which we used small and fast tumbling bicelles to study the conformation of the incorporated ligand, our present work of using uniformly aligned disk-shaped bicelles ($q = 2.7$ and total lipid concentration of 25%) demonstrates that they also allow us to obtain anisotropic NMR spectra encoding directional information. These unique and versatile features are, to our knowledge, not available in other model membrane systems. Thus, bicelle preparations can serve as versatile membrane systems to determine the conformation and orientation of a ligand in a membrane environment, information critical for understanding the interactions of lipophilic ligands with membrane proteins.

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Supporting Information Available: Additional dynamics simulation data. This material is available free of charge via the Internet at http://pubs.acs.org.

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